

CLAIMS

1. A DNA sequence, which codes for a polypeptide involved in the biosynthesis of cobalamins and/or cobamides.

5 2. The DNA sequence according to claim 1, which is chosen from

. the cobA, cobB, cobC, cobD, cobE, cobF, cobG, cobH, cobI, cobJ, cobK, cobL, cobM, cobN, cobO, cobQ, cobS, cobT, cobU, cobV, cobW, cobX and cobA genes presented

10 in Figures 15, 16, 40, 41, 47 and 52,

. homologues of these sequences resulting from the degeneracy of the genetic code, and

. sequences of natural, synthetic or recombinant origin which hybridise and/or which display significant

15 homologies with these DNA sequences or with fragments of the latter, and which code for polypeptides involved in the biosynthesis of cobalamins and/or cobamides.

20 3. A gene containing a DNA sequence according to one of the preceding claims.

20 4. Recombinant DNA, which contains at least one DNA sequence according to one of claims 1 to 3.

5. The recombinant DNA according to claim 4, wherein said DNA sequences are placed under the control of expression signals.

25 6. The recombinant DNA according to claim 5, wherein the expression signals may be homologous or heterologous to the DNA sequence.

7. The recombinant DNA according to one of claims 4 to 6, which forms part of an expression plasmid.

8. A plasmid, which contains at least one DNA sequence coding for a polypeptide involved in the biosynthesis of cobalamins and/or cobamides, and sequences permitting their expression.

9. The plasmid according to claim 8, which contains

10. . a recombinant DNA according to one of claims 4 to 7
. a replication system, and
. at least one selectable marker.

10. The plasmid according to claim 8, which is chosen from

15. . plasmid pXL1500 containing the cobA, cobB, cobC and cobE genes,
. plasmids pXL723 and pXL302 containing the cobC and cobD genes,
. plasmid pXL1397 containing the cobB and cobC genes,
20. . plasmids pXL368 and pXL557 containing the cobA and cobE genes,
. plasmids pXL545 and pXL545 Ω containing the cobE gene,
. plasmids pXL367 and pXL253 containing the cobF, cobG,
25. cobH, cobI, cobJ, cobK, cobL and cobM genes,
. plasmid pXL1148 containing the cobH and cobI genes,
. plasmid pXL1149 containing the cobH gene,

. plasmids pXL1496 and pXL1546 containing the cobF gene,

. plasmid pXL525 containing the cobA, cobE and cobF to cobM genes, plasmid pXL843 containing the cobX, cobS

5 and cobT genes, plasmid pXL699 containing the cobV gene, plasmid pXL1324 containing the cobU gene, plasmid pXL618 and pXL623 containing the cobQ and cobP genes, respectively, plasmid pXL593 containing the cobP and cobW genes and plasmid pXL1909 containing the cobP,

10 cobW, cobN and cobO genes.

11. A cell into which a DNA sequence according to one of claims 1 to 7 or a plasmid according to one of claims 8 to 10 has been introduced.

12. A polypeptide, which is involved in the biosynthesis of cobalamins and/or cobamides.

15 13. The polypeptide according to claim 12, which is encoded by a DNA sequence according to one of claims 1 to 7.

14. The polypeptide according to claim 12, which 20 participates in the conversion of precorrin-3 to 5'-deoxy-5'-adenosyl(Ado) cobyrinic acid a,c-diamide.

15. The polypeptide according to claim 14, which contains all or part of the COBB, COBF, COBG, COBH, COBJ, COBK, COBL, COBM, COBN, COBO, COBS and COBT 25 peptide sequences presented in Figures 15, 16, 40 and 41.

16. The polypeptide according to claim 14, which catalyses the transfer of a methyl group to positions C-1, C-5, C-11, C-15 or C-17 occurring between precorrin-3 and cobyric acid a,c-diamide.

5 17. The polypeptide according to claim 16, which contains all or part of the COBF, COBJ, COBL and COBM peptide sequences presented in Figure 16.

18. The polypeptide according to claim 12, which participates in the conversion of cobyric acid to
10 cobinamide.

19. The polypeptide according to claim 18, which contains all or part of the COBC and COBD peptide sequences presented in Figure 15.

20. The polypeptide according to claim 12, which
15 possesses an S-adenosyl-L-methionine:precorrin-2 methyltransferase (SP₂MT) activity.

21. The polypeptide according to claim 20, which contains all or part of the COBI peptide sequence presented in Figure 16.

20 22. The polypeptide according to claim 12, which possesses a cobyricic and/or hydrogenobyrinic acid a,c-diamide synthase activity.

23. The polypeptide according to claim 22, which contains all or part of the COBB peptide sequence
25 presented in Figure 15.

24. The polypeptide according to claim 12, which possesses a precorrin-8x mutase activity.

25. The polypeptide according to claim 24, which contains all or part of the COBH peptide sequence presented in Figure 16.
- 5 26. The polypeptide according to claim 12, which contains all or part of the COBE peptide sequence presented in Figure 15.
27. The polypeptide according to claim 12, which possesses a nicotinate-nucleotide:dimethylbenzimidazole phosphoribosyltransferase activity.
- 10 28. The polypeptide according to claim 27, which contains all or part of the COBU peptide sequence presented in Figure 41.
29. The polypeptide according to claim 12, which possesses a cobalamin-5'-phosphate synthase activity.
- 15 30. The polypeptide according to claim 29, which contains all or part of COBV peptide sequence presented in Figure 41.
31. The polypeptide according to claim 12, which possesses a cobyric acid synthase activity.
- 20 32. The polypeptide according to claim 31, which contains all or part of the COBQ peptide sequence represented in Figure 47.
33. The polypeptide according to claim 12, which possesses a cob(I)alamin adenosyltransferase activity.
- 25 34. The polypeptide according to claim 33, which contains all or part of the COBO peptide sequence presented in Figure 47.

35. The polypeptide according to claim 12, which possesses a precorrin-6x reductase activity.

36. The polypeptide according to claim 35, which contains all or part of the COBK peptide sequence presented in Figure 16.

37. The polypeptide according to claim 12, which participates in the conversion of cobinamide to GDP-cobinamide.

38. The polypeptide according to claim 37, which 10 possesses a cobinamide kinase and cobinamide phosphate guanylyltransferase activity.

39. The polypeptide according to claim 38, which contains all or part of the COBP peptide sequence presented in Figure 47.

40. The polypeptide according to claim 12, which 15 contains all or part of the COBS, COBT and COBX peptide sequences presented in Figure 40.

41. The polypeptide according to claim 13, which is chosen from the COBA, CORA, COBB, COBC, COBD, COBE, 20 COBF, COBG, COBH, COBI, COBJ, COBK, COBL, COBM, COBN, COBO, COBP, COBQ, COBS, COBT, COBU, COBV, COBW and COBX proteins presented in Figures 15, 16, 40, 41, 47 and 52.

42. A method for production of the polypeptides 25 according to claims 12 to 41, wherein

. a DNA sequence according to claims 1 to 7, or a plasmid according to claims 8 to 10 containing such a sequence, is introduced into a host cell,

5 . this recombinant cell is cultured under conditions for expression of the said sequence, and
. the polypeptides produced are recovered.

43. The method according to claim 42, wherein the host cell can be chosen from prokaryotes, eukaryotes and animal or plant cells.

10 44. The method according to claim 43, wherein the host cell is an archaebacterium or a eubacterium.

45. The method according to claim 44, wherein the host cell is E. coli, Pseudomonas denitrificans,
Rhizobium meliloti, Agrobacterium tumefaciens or
15 Salmonella typhimurium.

46. A method enabling the production of cobalamins and/or cobamides or of their precursors to be increased, wherein

20 . one or more DNA sequences coding for a polypeptide involved in the biosynthesis of cobalamins and/or cobamides is/are introduced into a microorganism productive of these compounds or potentially productive of these compounds,

25 . the microorganism thereby obtained is cultured under conditions for synthesis of cobalamins and/or cobamides and for expression of the said sequence, and

. the cobalamins and/or cobamides or their precursors produced are recovered.

47. The method according to claim 46, wherein one or more DNA sequences according to one of claims 4 to 5 7, or a plasmid according to claims 8 to 10 containing such sequences, is/are introduced into the microorganism.

48. The method according to claim 47, wherein the DNA sequence introduced into the microorganism codes 10 for a polypeptide catalysing a limiting step of the biosynthesis of cobalamins and/or cobamides.

49. The method according to claim 47, wherein the DNA sequences introduced into the microorganism code 15 for polypeptides catalysing limiting steps of the biosynthesis of cobalamins and/or cobamides.

50. The method according to one of claims 46 to 49, wherein the microorganism is chosen from P. denitrificans, R. meliloti and A. tumefaciens.

51. The method according to claim 50, wherein the 20 microorganism is P. denitrificans.

52. The method according to claim 51, wherein the microorganism is P. denitrificans SC510 Rif'.

53. The method according to one of claims 46 to 25 52, wherein a plasmid according to claim 10 is introduced into the microorganism.

54. The method according to claims 46 to 53,
wherein plasmid pXL525 is introduced into
P. denitrificans strain SC510 Rif'.

55. The method according to claims 46 to 54,
wherein the cobalamins and/or cobamides produced are
recovered by
. solubilisation,
. conversion to a cyano form, and
. purification.

10 56. The method according to claims 46 to 55,
wherein the cobalamin is coenzyme B₁₂.

57. The method according to claims 46 to 55,
wherein the precursor is chosen from
decobaltocorrinoids and corrinoids.